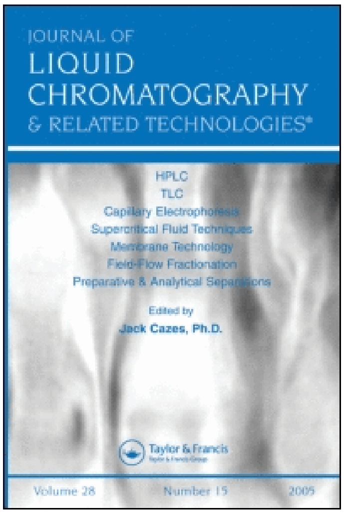

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SYNTHESIS AND CHARACTERIZATION OF A C_8 STATIONARY PHASE, BONDED WITH 2-ACRYLAMIDO-2-METHYL-1-PROPANESULFONIC ACID, FOR HPLC

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ABSTRACT

The silanization/hydrosilation method is used to bond a mixed stationary phase consisting of both octyl (C_8) and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) moieties. The intermediates, as well as the final product, are characterized by elemental analysis, diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy, and carbon-13 CP-MAS NMR spectroscopy. The results demonstrate that both the AMPS and C_8 groups are successfully bonded on the same silica hydride support material. The final product ($C_8 +$ AMPS) is characterized chromatographically and compared to the behavior of the same solutes on both a C_8 phase and an AMPS phase.

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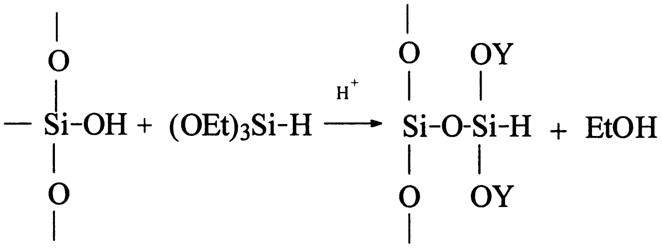
INTRODUCTION

The use of mixed stationary phases in HPLC can exploit the properties of each bonded moiety or functional group, or could eliminate some or all of the influence of the silica surface on solute retention. The latter approach would serve the same function as endcapping of the stationary phase. A number of investigations of mixed stationary phases have been reported over the last twenty years. Among the earlier investigations is the partial modification of aminopropyl silica with *n*-alkyl moieties of varying chain length.[1] Another study involved the synthesis of several phases with different ratios of cyano and undecyl ligands bonded to the surface.[2] A mixture of C_8 and cation exchange moieties was used for the analysis of pharmaceuticals.[3] Both C_8/cation exchange and C_8/anionic exchange phases were used for the separation of inorganic ions and neutral organics.[4] The combination of a strong anion exchange/C_{18} mixed mode stationary phase has also been used in capillary electrochromatography.[5] Restricted access stationary phases have been synthesized with mixed functional groups.[6] Another phase has been synthesized that contains both a hydrophobic chain and an imbedded urea group.[7,8] A mixed bed stationary phase containing amino and phosphoric acid was fabricated to be selective for organic compounds with polar functional groups.[9]

The silanization/hydrosilation bonding method is the synthetic approach used in this study.[10–12] It encompasses the following two reactions for the attachment of various organic moieties to silica surfaces:

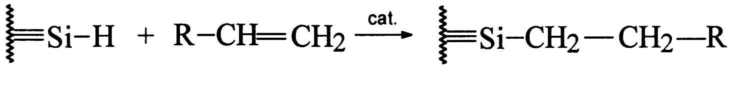
Silanization



$$-\underset{\substack{|\\ \mathrm{O}\\ |}}{\overset{\substack{|\\ \mathrm{O}\\ |}}{\mathrm{Si}}}\mathrm{{-}OH} + (\mathrm{OEt})_3\mathrm{Si{-}H} \xrightarrow{\mathrm{H}^+} \underset{\substack{|\\ \mathrm{O}\\ |}}{\overset{\substack{|\\ \mathrm{O}\\ |}}{\mathrm{Si}}}\mathrm{{-}O{-}}\underset{\substack{|\\ \mathrm{OY}}}{\overset{\substack{\mathrm{OY}\\ |}}{\mathrm{Si}}}\mathrm{{-}H} + \mathrm{EtOH}$$

Y = Si or H depending on the extent of crosslinking

Hydrosilation



$$\equiv\mathrm{Si{-}H} + \mathrm{R{-}CH{=}CH_2} \xrightarrow{\mathrm{cat.}} \equiv\mathrm{Si{-}CH_2{-}CH_2{-}R}$$

where cat = catalyst, typically hexachloroplatinic acid or free radical initiator.

The resulting product consists of organic moieties attached to the silica via silicon–carbon bonds with the remainder of the surface being predominantly Si–H groups. A few silanols can remain due to the fact that the silanization reaction is not 100% efficient in crosslinking. However, in most cases, the few remaining Si–OH groups are not accessible to any solutes when surface coverage is reasonably high ($>3.5\,\mu mol/m^2$) or the attached organic moiety is relatively large (such as octadecyl or larger). To fabricate a mixed phase by this synthetic method, two hydrosilation reactions are required in order to attach the two organic moieties. These reactions must be done consecutively since a mixed phase generally implies that the two organic groups have substantially different properties (such as one being relatively polar while the other is nonpolar). The feasibility of two consecutive hydrosilation reactions has already been demonstrated with the addition of an endcapping reagent to remove some of the excess hydrides on the silanized surface.[11] Consecutive reactions on a hydride surface have also been successful when a hydrosilation reaction was followed by organosilanization for endcapping.[13] In this study the possibility of two consecutive hydrosilation reactions with two bonded moieties of different polarity is investigated.

EXPERIMENTAL

Materials

The silica used in this study was Vydac TP 106 (The Separations Group, Hesperia, CA) with a surface are of $106\,m^2/g$, a particle size of 6.5 μm and a pore size of 300 Å. The 1-octene and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) for surface modification were purchased from Sigma-Aldrich (Milwaukee, WI). Triethoxysilane (TES) for the synthesis of the hydride intermediate was obtained from Huls Petrarch Systems (Bristol, PA). The catalyst, hexacholoroplatinic acid, was obtained from Strem Chemicals (Newburyport, MA) and the free radical initiator, azobisisobutyronitrile (AIBN) came from Sigma-Aldrich. Solvents for the syntheses and for the mobile phases were obtained in the highest purity possible. Two reversed phase test mixtures came from Perkin-Elmer (Nowalk, CT) and Supelco (Bellefonte, PA). Solutes for the other chromatographic tests were purchased from Sigma-Aldrich.

Instrumentation

Diffuse reflectance infrared Fourier Transform (DRIFT) spectra were obtained on a Mattson (Madison, WI) Infinity Series instrument interfaced to a

Venturis FX-2 computer. Carbon-13 cross-polarization (CP), magic-angle spinning (MAS) NMR spectra were recorded on a Varian (Palo Alto, CA) INOVA 400 MHz spectrometer system using a pulse width of 7 µs, a contact time of 1.5 ms and delay time of 3 s. Chromatographic measurements were made on either a Hewlett-Packard Model 1050 system (with ChemStation software) or a Perkin-Elmer System with a Series 200 pump, an LC 295 UV/Vis detector connected to a Hewlett-Packard 3396A integrator.

Synthesis of Bonded Phases

The hydride intermediate was synthesized according to the previously reported procedure.[10,12] Three bonded phases were produced by the hydrosilation reaction: C-$_{18}$, AMPS and C_{1B}- endcapped with AMPS. The general procedures for bonding terminal olefins to hydride surfaces have been reported previously.[11,12] Some changes were made to accommodate the endcapping process and the use of a polar bonded group, AMPS. For endcapping the C_{18}-material, the hydrosilation reaction with AMPS utilized ethanol as the solvent at a concentration of 0.75 mg/mL. The reaction time was 90 hours at a temperature of 68°C. Both hexachloroplantic acid and AIBN were tested as possible catalysts for hydrosilation of AMPS.

The bonded phase surface coverage was determined by carbon analysis (Desert Analytics, Tucson, AZ) and calculated by an equation modified for terminal olefin bonding on a hydride surface.[14] The products were packed in standard 150×4.6 mm stainless steel columns (Alltech, Deerfield, IL) for chromatographic evaluation.

RESULTS AND DISCUSSION

The results of the surface coverage (in µmole/m^2) for the three phases synthesized in this study, as determined from carbon analysis (after correction for the 0.3% carbon on the hydride intermediate), are as follows: $C_{18} = 3.87$; $C_{1B} + \text{AMPS} = 6.38$; $\text{AMPS} = 7.79$. The value for the octadecyl material is reasonable for a typical monomeric stationary phase. However, the values for the AMPS and C_{1B} endcapped with AMPS are generally much higher than can be obtained if only monomeric bonding occurred. This result suggests that some polymerization of the triethoxysilane occurred in the formation of the hydride layer on the silica surface. Therefore, the total number of hydrides available for bonding would be greater than the total number of silanols on the original silica surface. Successful bonding of AMPS was obtained by either hydrosilation with Speier's catalyst or free radical addition using AIBN.

In order to better characterize the modified surface, both DRIFT and carbon-13 CP-MAS spectra of the three phases were obtained. In addition, the DRIFT spectrum of the hydride intermediate was acquired and it displayed the strong Si—H stretching band at $2251\,cm^{-1}$ as reported previously.[10] The DRIFT spectrum of the octadecyl material is characterized by a broad C—H stretching band with a maximum near $2951\,cm^{-1}$and an Si—H band that is diminished in intensity when compared to the hydride intermediate spectrum. The DRIFT spectrum of the AMPS phase has distinct peaks at 2683 and $2571\,cm^{-1}$, as well as a diminished Si—H peak, when compared to the hydride intermediate. The spectrum of the C_{1E} phase endcapped with AMPS has, as expected, features of the spectra of each compound described above. Thus, strong peaks are seen at 2951, 2683, and $2571\,cm^{-1}$. The results are the same whether endcapping by AMPS occurs via hydrosilation or free radical addition.

Further insight into the nature of the various bonded phases can be gained from examination of the carbon-13 CP-MAS NMR spectra. Figure 1(A) shows the spectrum of the C_8 bonded material. The peak near 12 ppm represents the methylene group attached to a silicon atom on the surface. The peak at 23 ppm is due to the methyl group of the C_8 moiety, and the remainder of the methylene groups are found in the peaks between 24 and 36 ppm. Figure 1(B) is the spectrum of the AMPS bonded material. The key peak in this spectrum is at 15 ppm that represents the carbon in the Si—C linkage at the surface. Another interesting feature of the spectrum are the two peaks between 25 and 30 ppm that represent the two methyl groups in AMPS. The two methyl groups are not equivalent due to the restricted motion in the solid state caused by the presence of carbonyl and sulfoxide moieties. The chemical shifts for the various carbons, and the uniqueness of the methyl groups in the solid state, can be documented by the spectra of the starting alkene (2-acrylamido-2-methyl-1-propanesulfonic acid) as a solid and in solution. Figure 2(A) shows the carbon-13 spectrum of AMPS dissolved in D_2O. Here it can be seen, that in solution the methyl groups rotate freely resulting in a single peak at 26 ppm. The other peaks can be easily assigned as follows: the methylene group at 52 ppm; the quaternary carbon at 57 ppm; the two alkene carbons at 127 and 131 ppm; and the carbonyl group at 167 ppm. When the spectrum [Fig. 1(B)] of the same compound is taken as a solid, the peaks observed can be correlated well with those of the liquid sample. Some additional peaks are present in the spectrum that could be due to either sidebands or multiple chemical shifts due to hindered rotation in the solid. As is the case with the bonded AMPS moiety, the methyl groups are not equivalent and two peaks are observed at 25 and 29 ppm. The other peaks correspond reasonably well to the solution spectrum with values at 53, 55, 126, 133, and 168 ppm. The remainder of the peaks in Fig. 1(B) are the two methylene groups adjacent to the carbonyl and sulfoxide

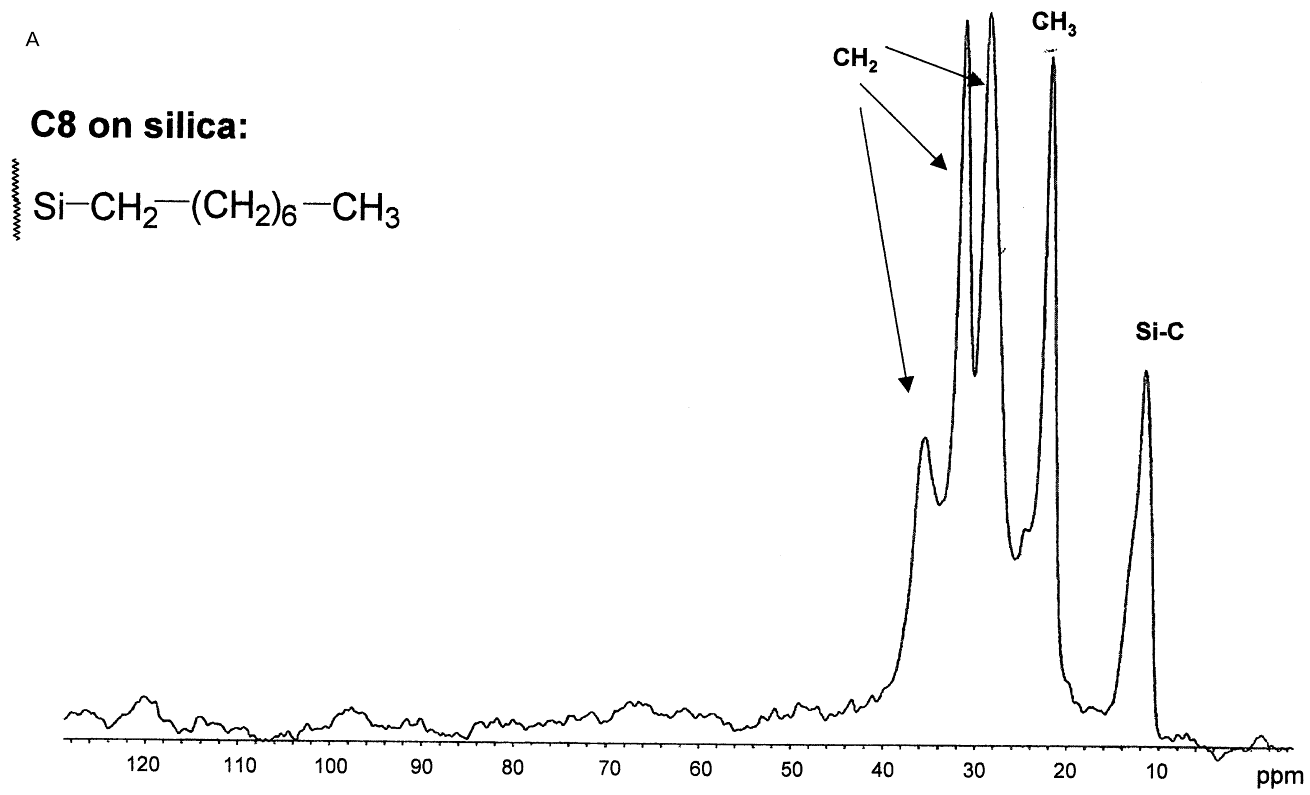
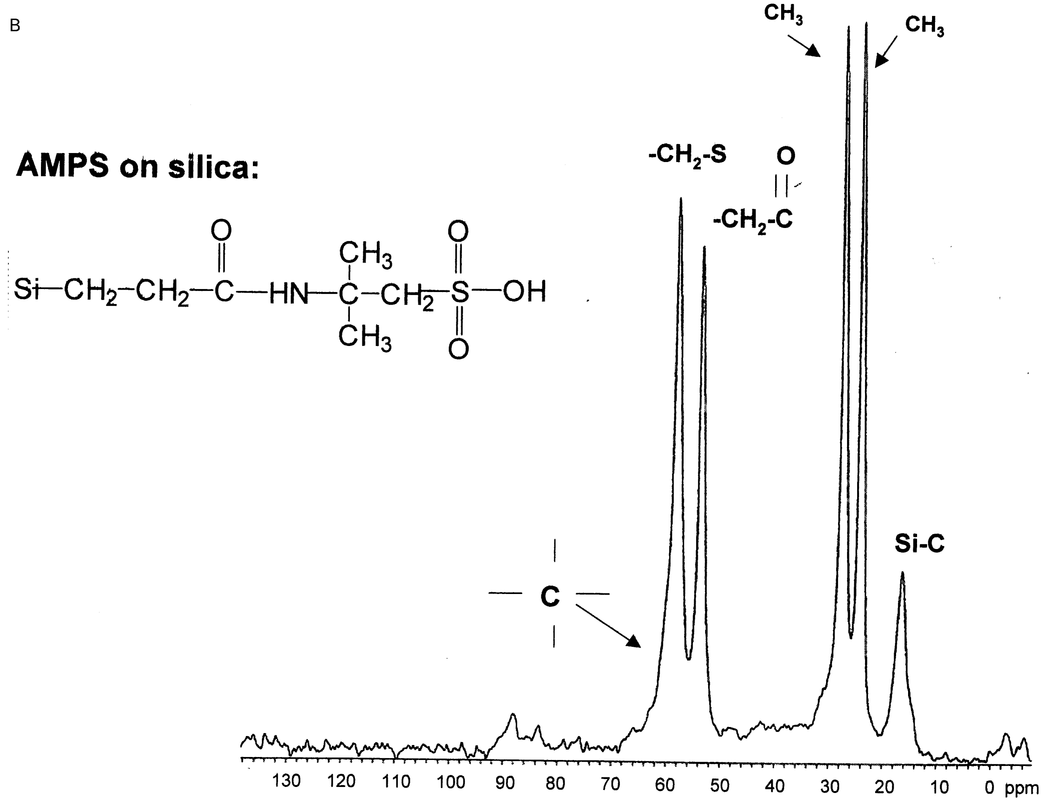


Figure 1. Carbon-13 CP-MAS NMR spectra of bonded phases evaluated in this study. (A) C_B bonded material; (B) AMPS bonded material; and (C) C_8 bonded material endcapped with AMPS.



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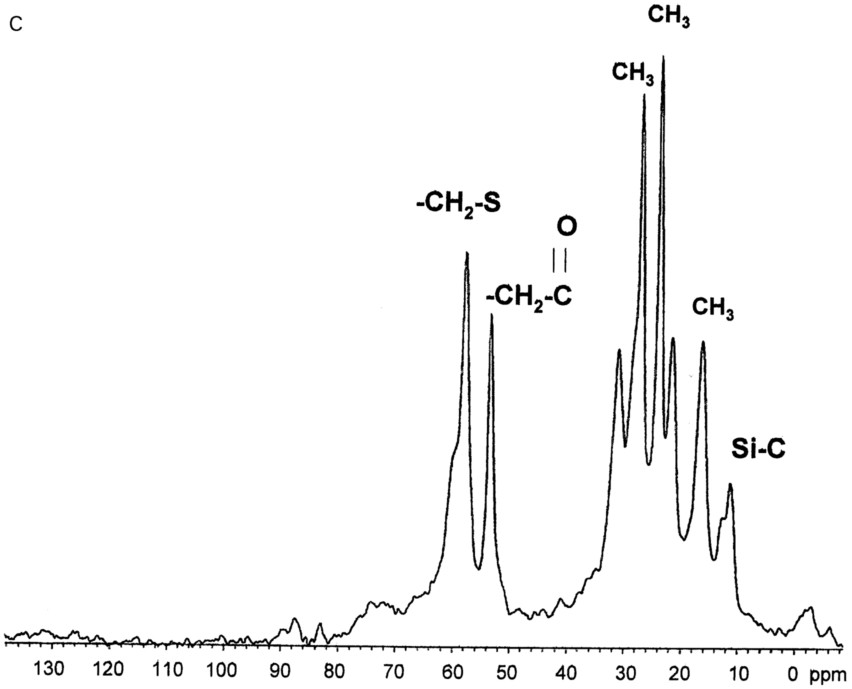

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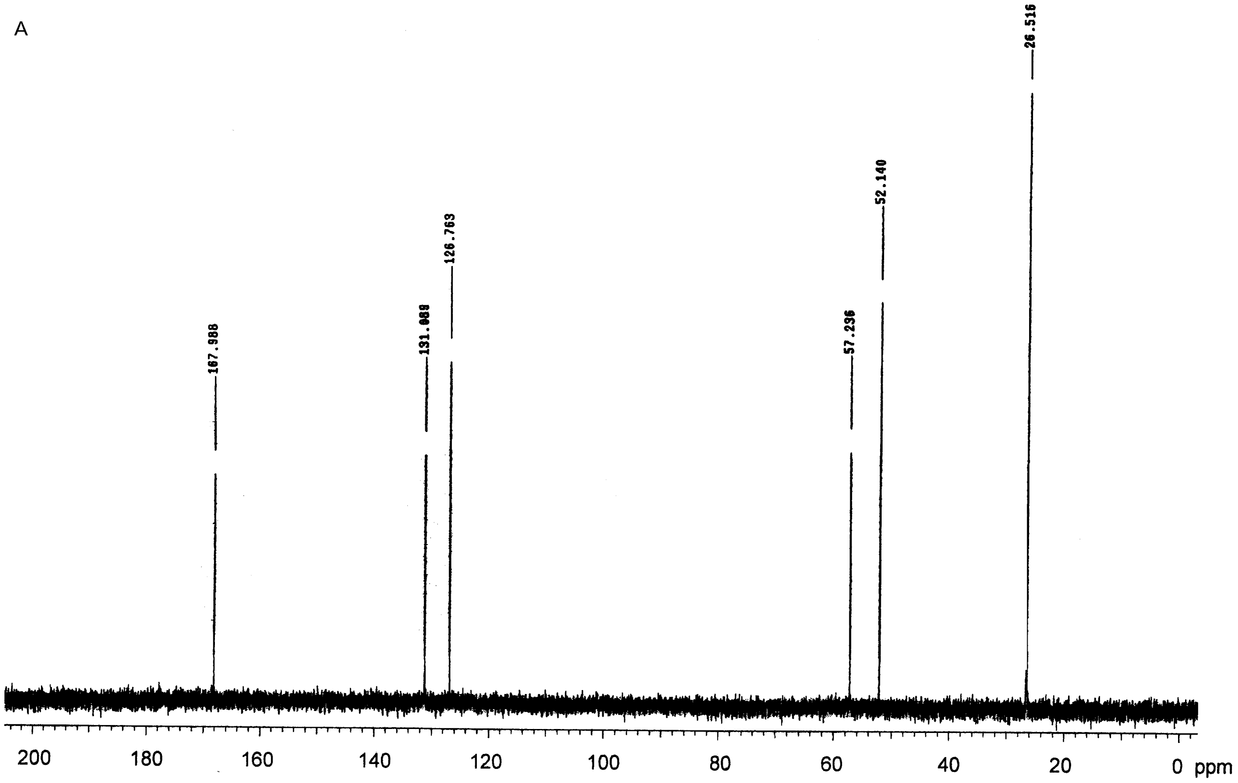
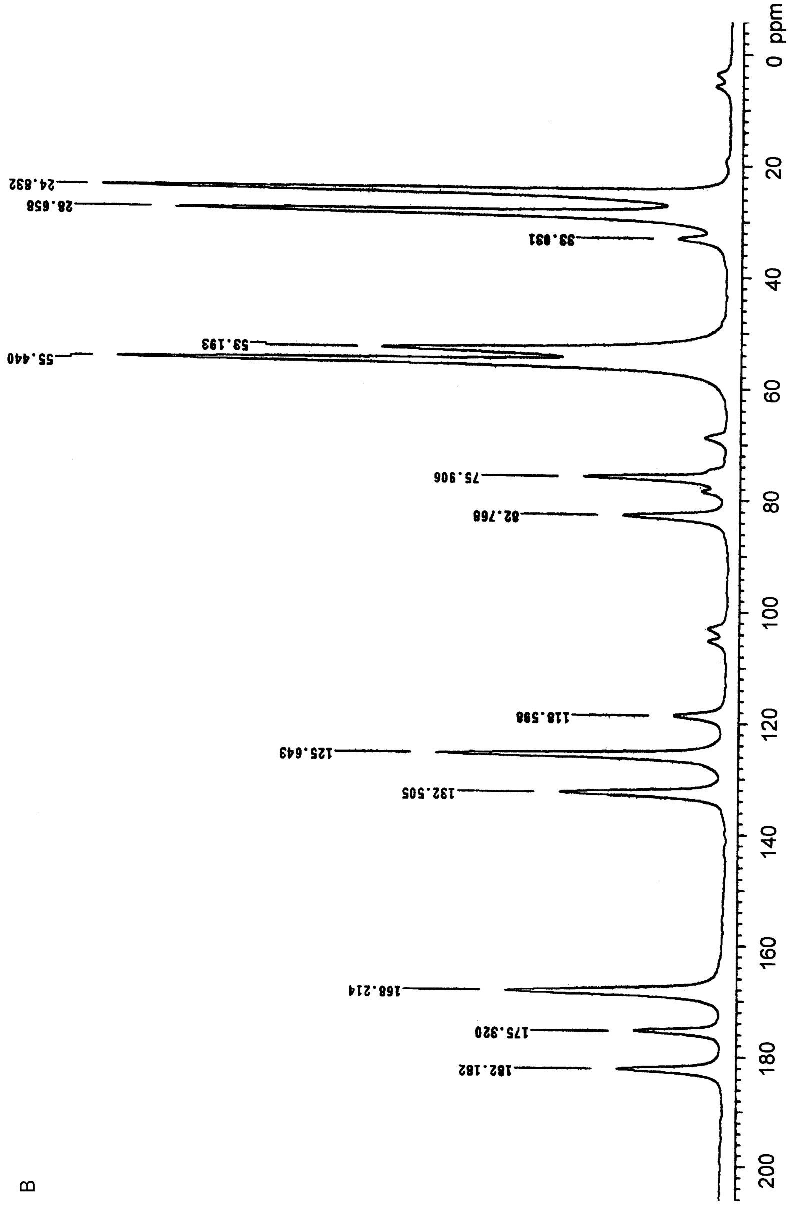


Figure 2. Carbon-13 NMR spectra of AMPS starting material. (A) solution spectrum of AMPS in D_2O and (B) solid state CP-MAS spectrum.

(*continued*)



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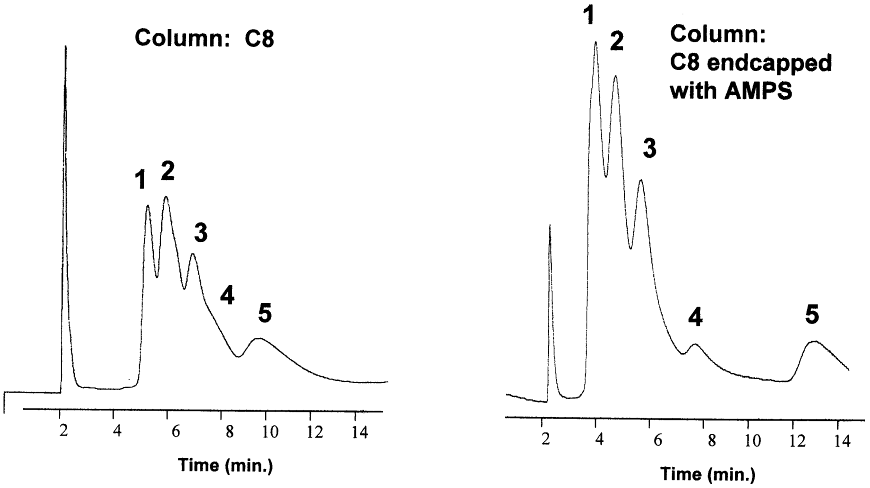

Figure 3. Separation of tetracycline mixture on the C_B column and the C_8 endcapped with AMPS column. Mobile phase 90 : 10 acetonitrile/water. Flow rate = 0.5 mL/min. Detection at 280 nm. Solutes: 1 = oxytetracycline; 2 = minotetracycline; 3 = democyline; 4 = chlorotetracycline; and 5 = doxycline.

moieties, respectively, and the quaternary carbon that are between 50 to 60 ppm. Not shown, is the peak for the carbonyl carbon at 168 ppm. Finally, the success of producing the mixed phase is documented in Fig. 1(C). Clearly, the peaks described above for the C_8 and AMPS phases, individually, can be seen in the spectrum of the mixed phase.

Further characterization of mixed phase was done chromatographically, using a number of test solutes to probe the properties of this material. All of the phases synthesized were able to separate under reasonable sets of conditions, including mixtures of small hydrophobic solutes such as benzene, toluene, ethylbenzene, isopropylbenzene, butylbenzene, anthracene, and acetophenone. In all cases, peak symmetry was good and retention was typical reversed phase behavior, with retention decreasing as the amount of organic (methanol) constituent in the mobile phase increased. More significant differences can be observed for a number of basic compounds. Figure 3 shows a comparison between the C_E and C_8 endcapped with AMPS columns for the separation of a mixture of tetracyclines. Under identical mobile phase conditions, the C_8 column endcapped with AMPS has longer retention, higher resolution, and better peak shape than the C_8 columns. Both the higher carbon load of the endcapped material leading to a better-protected surface, and the more polar nature of the final phase, can account for the difference in column performance.

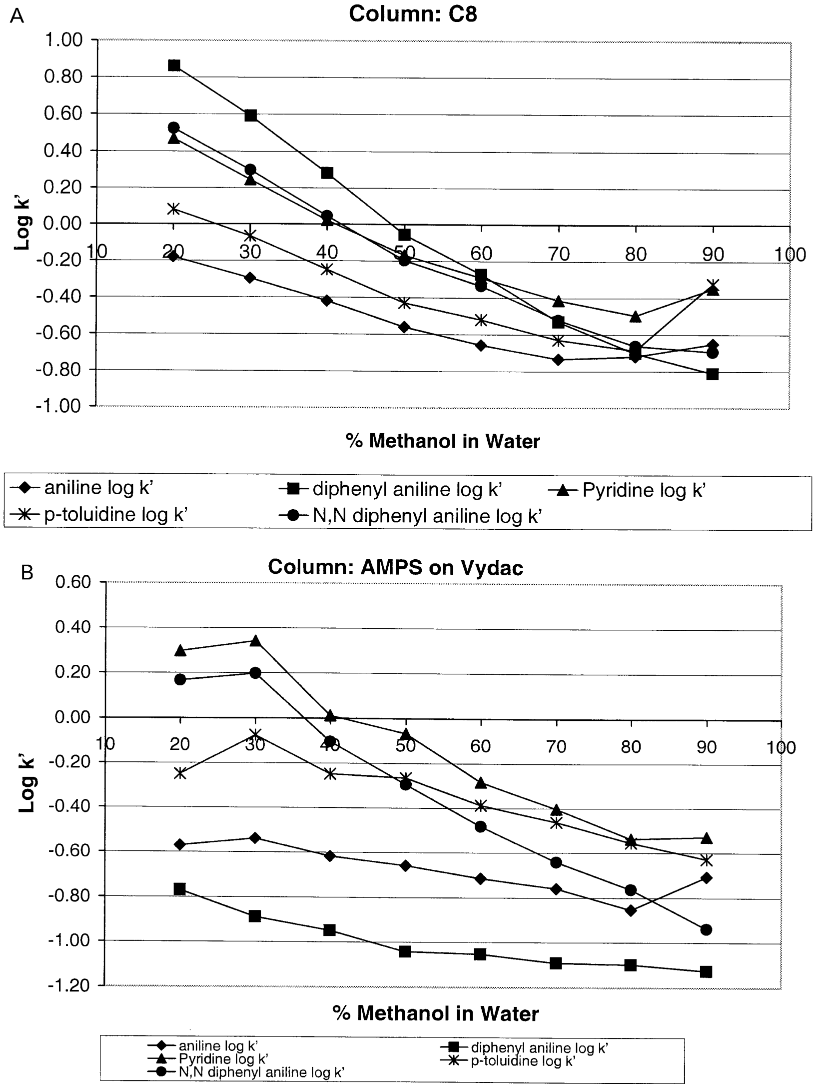

Figure 4. Log k' vs. % methanol in the mobile phase. (A) C_8 column; (B) AMPS column; and (C) C_8 endcapped with AMPS column. Flow rate = 0.5 mL/min. Detection at 254 nm.

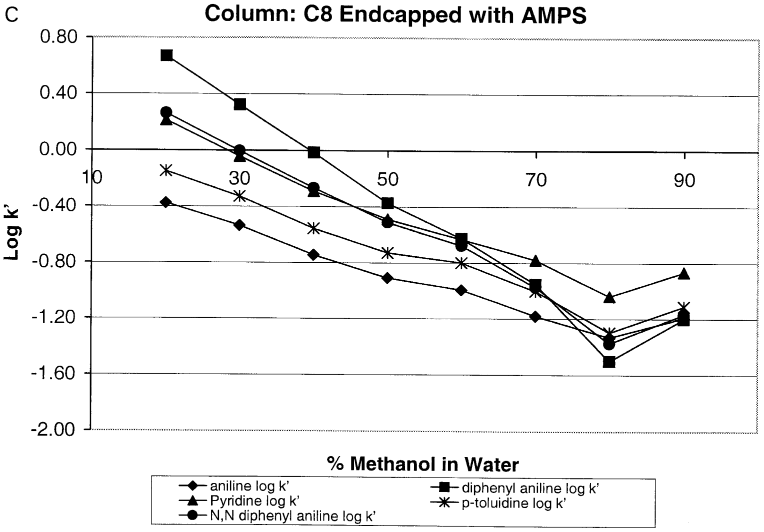

Figure 4. Continued.

Another evaluation of the retention characteristics of the C_8 phase endcapped with AMPS, can be made by plotting log k' vs. % methanol in the mobile phase for a number of different solutes. This data can then be compared to the results obtained for C_8 and AMPS columns. The results of this comparison are shown in Fig. 4. The C_B column [Fig. 4(A)] displays typical reversed phase behavior. Log k' generally decreases with increasing MeOH in the mobile phase except at very high percentages of the organic component. Some of the test solutes show a small increase in log k' under these conditions. In contrast, the AMPS column displays a much more irregular behavior over the same range of mobile phase compositions [Fig. 4(B)]. The C_8 column endcapped with AMPS [Fig. 4(C)], shows a behavior that is very similar to that observed for the C_8 column, indicating that the predominant mode of retention is a reversed phase mechanism.

The final test of the potential usefulness of the endcapped phase involves the peak symmetry of several solutes. Figure 5 shows plots of the asymmetry factor (A_s) for four compounds as a function of the percent methanol in the mobile phase. All of these test solutes are basic and could strongly interact with residual silanols on the surface. The results for all four species are consistent with the endcapped column, have the highest symmetry followed by the C_B and finally the AMPS column. The log k' data clearly shows that the C_g group controls retention when a mixed phase is present. Endcapping the C_8 material improves

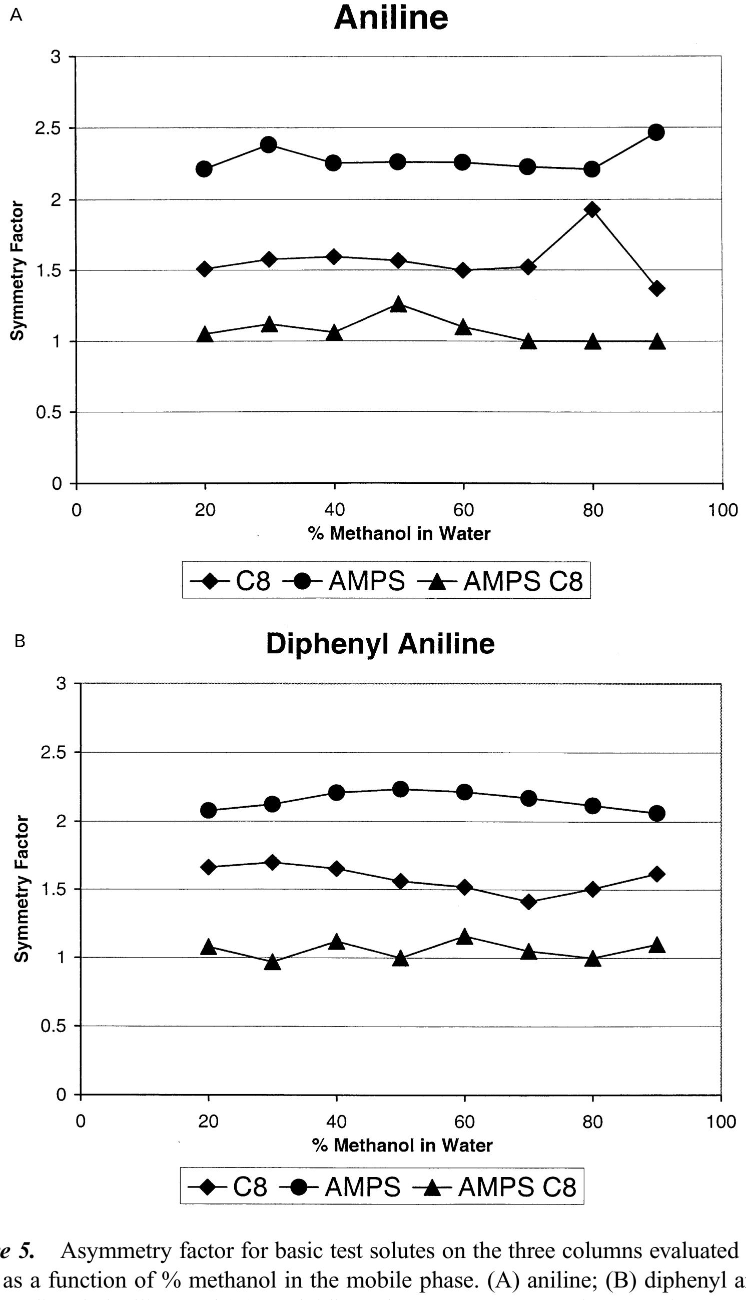

Figure 5. Asymmetry factor for basic test solutes on the three columns evaluated in this study as a function of % methanol in the mobile phase. (A) aniline; (B) diphenyl aniline; (C) *N*, *N*-dimethylaniline; and (D) *p*-toluidine. Flow rate = 0.5 mL/min. Detection at 254 nm.

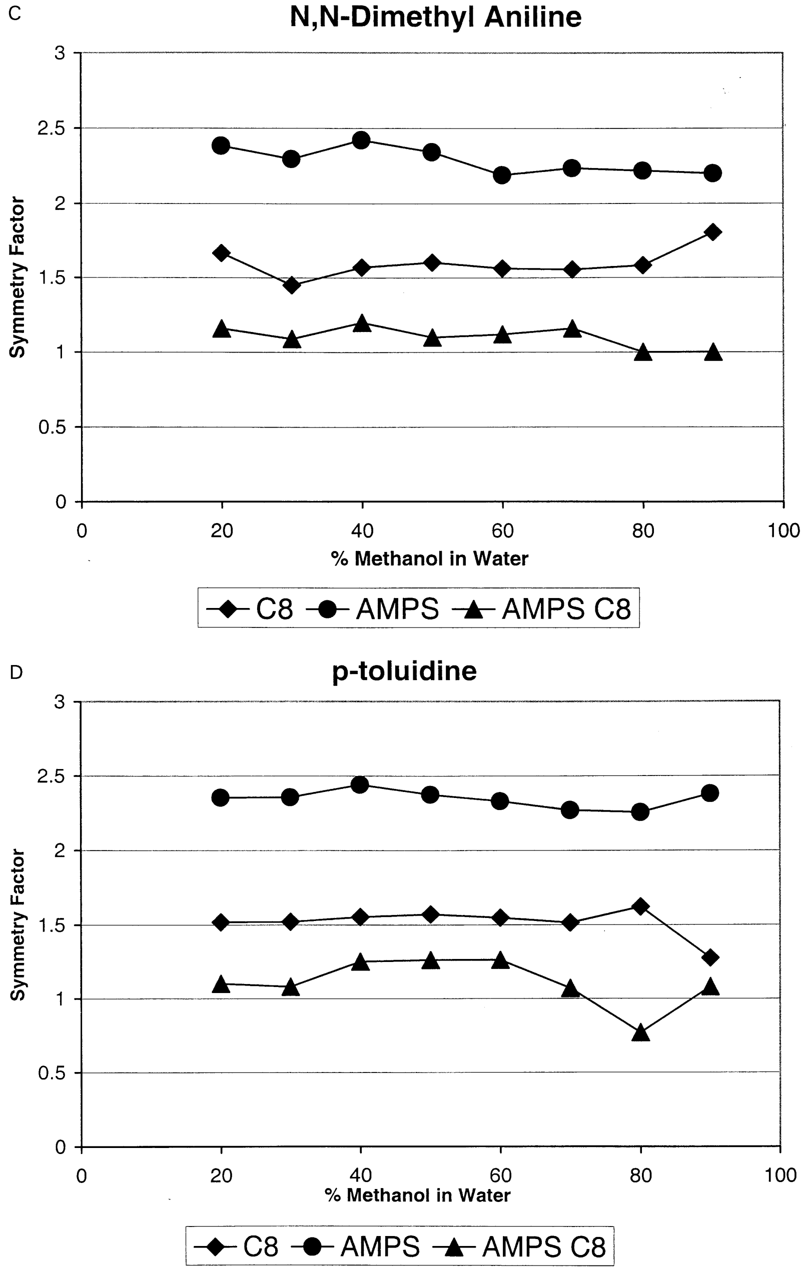

Figure 5. Continued.

the symmetry, as shown in Fig. 5 and leads to somewhat longer retention, as shown in Fig. 4. The slightly poorer symmetry on the AMPS may be due to the lower degree of shielding of the surface than the mixed phase, since it is a relatively small moiety, or it could be due to polar interactions between the solutes and AMPS. These polar interactions could be masked in the mixed phase by the more dominant hydrophobic effect of the C_8 group, as indicated in the retention data.

CONCLUSIONS

The successful synthesis of a mixed phase, based on the combination of a nonpolar group (C_8) followed by endcapping with a polar entity (AMPS) using the silanization/hydrosilation method, has been demonstrated. The presence of both groups on the surface has been confirmed by DRIFT and carbon-13 CP-MAS NMR spectra. The new material has properties that could be useful for the separation of basic compounds, as shown in the separation of tetracyclines and the peak symmetry measurements for a number of compounds over a broad range of mobile phase compositions. Further evaluations will be directed toward pharmaceutical and biological applications.

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